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Peptide supplementation to nutrient-adequate diets enhanced internal egg quality during storage in hens at peak production

Running title: Dietary peptide effect on egg production and quality

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ABSTRACT

BACKGROUND: There is paucity of information on the use of dietary peptides in laying hens and its effects on egg production and quality. In the current study, peptide from enzymatic hydrolysis of soybean protein was incorporated into laying hens diets to investigate its effect on egg production and internal egg quality.

RESULTS: There were no treatment effects on egg production (average hen day production was 96%) during the experiment. Final body weight of the hens increased quadratically ($P < 0.05$) in response to peptide supplementation. There were no significant effects of peptide supplementation on internal egg quality of the fresh eggs. Peptide supplementation tended to increase yolk color ($P < 0.10$) in eggs collected at 4 weeks of the study and stored at room temperature for 14 days. For the eggs collected at 8 weeks of the experiment and stored at room temperature for 14 days, peptide supplementation linearly increased ($P < 0.05$) albumen height, Haugh unit and yolk index but linearly decreased ($P < 0.01$) yolk width.

CONCLUSION: Peptide supplementation to laying hens at peak production, receiving diets meeting their nutrient requirement, did not improve hen production but positively helped to maintain hens' body weight and egg quality during storage.

Keywords: enzymatically hydrolysed soybean protein, peptides, hens, egg production, egg quality

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INTRODUCTION

Maintaining adequate protein and energy levels is essential to ensuring optimum laying performance and maintenance of body condition of hens during lay.[1,2] The objective of supplying protein is mainly to provide the amino acids however the presence of anti-nutritive factors in common feedstuffs may limit the use of intact protein in these feedstuffs and may increase nitrogen output to the environment.[3] Peptides, which are hydrolytic products of enzymatic or fermentation hydrolysis of proteins are very rapidly translocated in the intestine.[4] In view of the greater effectiveness of peptides compared to intact protein, they can help to more effectively meet animal amino acid needs and reduce nitrogen excretion to the environment.

Dietary supplementation of peptides may help support better growth performance and intestinal health than the feeding of intact protein such as soybean meal or fish meal.[5,6] Studies with fish larvae have shown the promise of peptide feeding in supporting more optimum growth performance.[5,7]. Those effects are likely due to the more optimal utilization of peptides compared to intact proteins[8]. The use of fermented soybean has also been shown to be effective in enhancing growth performance and gut health of piglets and broilers.[9-11] It can be reasoned that the positive effects of these fermented protein sources on gut health and performance can ultimately improve the quality of animal products. As previously noted for pigs and broilers, it can be expected that replacing part of intact protein in layer diets with peptides will also result in enhanced productive performance and product quality of the hens. However there is paucity of data on feeding of peptides to layers generally or of its effects on egg quality specifically. Therefore the objective of the current experiment was to study the impact of part replacement soybean in laying hen diets, with graded levels of soybean peptide, on egg production, performance during lay and egg quality of hens during the first phase of lay (peak production phase).

EXPERIMENTAL

All the animal experiment procedures in the current study were approved by the Scotland's Rural College Animal Experiment Committee.

Peptide

The peptide (Fortide®) used in the current experiment was derived from enzymatic hydrolysis of soybean protein. The production process of Fortide is as follows: the raw material was based on a certain proportion of dehulled solvent extracted soybean meal (SBM) and soy protein concentrate (SPC). The SBM and SPC were sterilised at 105℃ for 30 min, and then cooled to 50°C. The sterilised product was mixed with calcium hydroxide and alkaline protease, hydrolysed at 1:4 wt vol^{-1} in distilled water, at 50°C and pH 8.0-9.0 for 10 h. The intermediate product after enzyme hydrolysis was subsequently dried at 90°C for 12 h. The chemical composition of the peptide is shown in Table 1.

Birds, feed and management

A total of 800 Lohmann Brown laying hens at 24 weeks old were used for the experiment to study the impact of dietary inclusion of graded levels of a peptide (Fortide®, MyTech Biotech Co., Ltd, PR China) on egg production, hen performance and internal egg quality during the first phase of lay. At the start of the experiment, the hens were allocated to four treatments. Each of the treatments had 10 replicate cages with 20 hens per replicate cage. The cages were enriched colony cages, and each cage was equipped with a nest, scratch mat and perches. No additional furniture was provided in the cages. The hens were allocated to cages to ensure that the initial body weight of hens was similar across treatments. The hens were housed in a facility that enabled regulation of temperature and humidity.

All the birds received diets (in mash form) that were formulated to meet the nutrient requirement according to nutrient specification for Lohmann brown hens (Table 2). The control diet was without any peptide supplementation whereas the remaining three diets were supplemented with 2, 4 or 8 g kg^{-1} of the peptide. Feed and water were provided in ad libitum basis for the duration of the experiment. Birds were weighed at the beginning and end of the experiment whereas feed intake was monitored on biweekly basis.

Egg production

Total eggs laid were collected and weighed daily to determine egg mass and for calculation of hen day production. After weighing, eggs with defects (small, shell less, deformed, cracked) were noted and recorded.

Egg quality assessment

All the eggs produced per cage were collected at the end of weeks 4 and 8 of the study. Six eggs were randomly selected from the eggs produced per cage and were used for internal egg quality assessment. Three of the six randomly selected eggs were used for egg quality assessment on the day of collection (fresh eggs). The remaining three eggs per cage were stored on the bench in the laboratory (stored eggs) at room temperature (approximately 14℃) for 14 days before egg quality analysis was done on them. The procedure was repeated on eggs collected at the end of week 8 of the study. Consequently a total of 30 eggs were used per treatment at each egg quality assessment.

All the egg quality assessments were done using egg quality equipment QCM+ ™ and QCC ™ (Technical Services and Supplies Ltd, York, United Kingdom). The egg qualities assessed by the equipment were albumen height, Haugh unit and yolk colour. Yolk height and yolk width were manually measured using Vernier calliper whereas yolk index[12] was calculated from data of yolk height and width using the relation: $Yolk\ index = \frac{Yolk\ width + Yolk\ width\ at\ 90°}{Yolk\ height}$.

Chemical analysis

The diets were analysed for dry matter, crude protein and minerals using Association of Official Analytical Chemists (AOAC) procedures. Albumen was collected from eggs sampled at the end of weeks 4 and 8 of the experiment. The albumen was then lyophilized and subsequently analysed for crude protein content. Albumen crude protein content was expressed on dry matter basis.

Statistical analysis

All the data were analysed using the GLM procedure of SAS (2013) as randomised complete block design. Statistically significant means were separated using orthogonal polynomial contrasts.

RESULTS

Table 3 shows the egg production data during the eight weeks of the study. There were no treatment effects on egg production during any of the phases in the experiment. The hen day production averaged 96% and average egg weight was 58.9 g during the 8 weeks of the study.

Table 4 shows the effect of the treatment on feed intake, FCR and weight gain of the hens during the experimental period. There were no significant treatment effect on feed intake and egg FCR at any phase or the overall study period. Final body weight of the hens increased quadratically ($P < 0.05$) in response to peptide supplementation.

Table 5 shows the data on internal egg quality after feeding diets supplemented with peptide for four weeks. There were no significant effect of peptide supplementation on any of the internal egg quality assessed.. The yolk color for eggs from hens receiving peptide-supplemented diet tended ($P < 0.10$) be greater in eggs stored in room temperature for 14 days after collection.

The data on internal quality of eggs from hens receiving peptide-supplemented diet for 8 weeks are shown in Table 6. For the eggs assessed on the day of collection, peptide supplementation had no significant effect on any of the internal egg quality assessed. In the eggs stored at room temperature for 14 days, peptide supplementation linearly increased ($P < 0.05$) albumen height, Haugh unit and yolk index. On the other hand, peptide supplementation linearly decreased ($P < 0.01$) yolk width in eggs stored in room temperature for 14 days.

DISCUSSION

The objective of the current experiment was to study the influence of a peptide supplementation to hen diets on egg production and egg internal quality at collection and during storage. Collection of data at 4 and 8 weeks after the onset of feeding of experimental diets enabled comparison of the response to peptide supplementation at these different periods. The diets were formulated to meet the nutrient requirement of Lohmann brown and were all based on a control treatment that was without peptide. The peptide (Fortide) was derived from enzymatic hydrolysis of soybean protein and added at graded levels to nutrient-adequate diets.

There are advantages to the use of peptide instead of intact protein because peptide transporter can more efficiently, from energetics points of view, transport 2- or 3-amino acid peptides than single amino acid.[4,13] From animal nutrition point of view, it has been suggested that feeding of peptides or fermented or enzymatically hydrolyzed protein feedstuffs improved growth performance, is an effective way of meeting amino acid requirements in low-protein diets and influences endogenous amino acid loses.[14-16] Aside from nutritional effects though, peptides also serve functional food roles and help to enhance gut health[7] or reduce stress response.[17,18] However there is a scarcity of reports on the supplementation of peptides in poultry diets.

The observation from the current study showed that there were no effects of supplementation of the peptide on egg production performance of the hens. This lack of effect was probably due to several reasons. First the hens were at peak production and clearly at such high production in the control diet, there was hardly any room for improvement due to peptide supplementation. Secondly the diets were adequate in nutrient and hence in the absence of intense stress factor that could have impeded nutrient utilization there was hardly any negative effects that feeding of the peptide could potentially alleviate.

There were effects of peptide supplementation on internal quality of eggs without, as previously noted, any effect on production. Similar observation had been made in other studies.[19] It was observed that effects of peptide supplementation on internal quality of eggs were mainly observed after 8 weeks of feeding the experimental diets. This may suggest that it takes longer than 4 weeks for the peptide to exert its effect on egg quality. This apparent latent period for the peptide supplementation effect may depend on the nutritional status of the birds at the beginning of the trial period. It is reasonable to assume that provision of peptide supplementation prior to lay may be a way of ensuring that the positive effects of the additive are realized when egg laying starts.

Generally the positive effect of peptide supplementation on egg quality was observed in eggs that have been stored for two weeks in room temperature. It is well documented that eggs quality normally deteriorates in storage[20,21] with deterioration rate dependent on temperature and moisture content of the place where eggs are stored. The positive effect of the peptide was in helping to reduce deterioration of egg quality during storage and thus helping to extend egg shelf life in room temperature. Eggs from hens receiving peptide supplementation had higher albumen height and Haugh Unit. Haugh unit reflects the thick albumen content of the egg and the viscosity of the albumen reflects its ovomucin content.[22] Generally egg albumen becomes more watery (less viscous) during storage and this has negative consequence of lowering protein structural integrity,[23] albumen height and consequently Haugh unit. It is possible that protein quality, if not content, of the albumen deteriorates during egg storage[20] and that peptide supplementation protected against that effect. This agrees with the nutritional importance of dietary protein in maintain egg albumen quality.[24]

There were also protective effects of peptide supplementation on the yolk. The yolk width was reduced with increasing level of peptide supplementation in eggs stored in room temperature for two weeks. This reasonably is indicative of strength of the vitelline membrane surrounding the yolk. Vitelline membrane that has lost its integrity allows for greater stretching leading to yolk with greater width and more prone to breakage. An uncompromised vitelline membrane is an important quality in the egg-breaking industry as it helps to prevent the contamination of the albumen with yolk. As eggs age, the loss of o-glycosidically linked carbohydrate units of glycoprotein causes both egg white thinning and compromised integrity of vitelline membrane[25,26] leading to deterioration of albumen and yolk membrane quality.

The hens receiving peptide supplementation in the current study had greater final body weight compared with those on the control diet. It has been reported that peptide supplementation influenced peptide transport and this may have overall effect on protein utilization,[27] possibly also influencing composition of gain. The composition of gain or the specific component where the gain preferentially occurred during the study was not investigated but maintenance of weight during egg production is important to ensure that high level of production during lay is ensured.

CONCLUSION

Peptide supplementation to nutrient-adequate diets helped to maintain egg internal quality during storage and helped the hens to maintain their body weight during early laying phase. Because of the reported positive effects of peptide, it is possible that supplementation of the additive in later phases of laying will help hens to maintain high production while also helping to maintain egg internal quality during storage. This aspect needs to be further investigated.

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Table 1. Ingredient and chemical compositions (g kg^{-1}) of the experimental diets

Items	Diet numbers			
	1	2	3	4
Wheat	544.6	544.6	544.6	544.6
Maize	100.0	100.0	100.0	100.0
Soyabean meal 48%	215.0	213.0	211.0	207.0
Soya oil	30.0	30.0	30.0	30.0
DL methionine	1.40	1.40	1.40	1.40
Calcium carbonate	82.0	82.0	82.00	82.0
Dicalcium phosphate	12.0	12.0	12.00	12.0
Salt	1.80	1.80	1.80	1.80
Bicarbonate Sodium	2.90	2.90	2.90	2.90
Yellow carophylle	0.03	0.03	0.03	0.03
Lucantin red 10%	0.02	0.02	0.02	0.02
Ethoxyquin 66%	0.20	0.20	0.20	0.20
VTM premix*	10.0	10.0	10.0	10.0
Phyzyme XP TPT	0.05	0.05	0.05	0.05
Fortide	0.00	2.00	4.00	8.00
Total	1000.0	1000.0	1000.0	1000.0
Calculated nutrient composition				
Crude protein†	170.0	164.4	165.0	177.5
Metabolisable energy	11.8	11.8	11.8	11.8

MJ kg^{-1}				
Ca†	30.4	33.0	29.0	34.3
P†	4.7	4.4	4.7	4.8
Avail P	3.0	3.0	3.0	3.0
Met	3.9	3.9	3.9	3.9
Cys	3.0	3.0	3.1	3.1
Met + Cys	7.0	7.0	7.0	7.0
Lys	8.2	8.2	8.2	8.2
His	4.3	4.3	4.3	4.3
Trp	2.2	2.2	2.2	2.2
Thr	5.9	5.9	5.9	5.9
Arg	10.5	10.5	10.5	10.6
Ile	6.7	6.7	6.7	6.7
Leu	12.5	12.5	12.5	12.4
Phe	13.2	13.2	13.2	13.1
Val	7.6	7.6	7.6	7.6

* VTM (Vitamin and trace minerals) premix provided per kilogram of diet: Mn, 80 mg; Zn, 60 mg; Fe, 10 mg; Cu, 5 mg; Se, 0.15 mg; I, 1.0 mg; vitamin A, 6,000 IU; vitamin D3, 3,000 IU; vitamin E, 5 IU; vitamin B12, 25 µg; vitamin K, 1.0 mg; niacin, 10 mg; folic acid, 0.30 mg; pantothenic acid, 4 mg.

† Analysed content

298 **Table 2.** Chemical composition (g kg^{-1}, as is) of the peptide used in the experimental diets

Item	Composition
Acid soluble protein	285
Dry matter	920
Crude protein	≥ 460
Crude ash	≤ 150
Crude fat	25
Crude fiber	≤ 70
Nitrogen free extract	235
Ca	33
P	10
Amino acids	
Val	19.5
Leu	2.62
Ile	16.0
Met	9.4
Cys	8.0
Phe	21.0
Tyr	12.8
Trp	6.0
Arg	41.2
Lys	30.6

His	12.0
Thr	14.2
Gly	17.3
Ala	17.5
Pro	22.7
Ser	23.5
Asp	58.5
Glu	93.0

Table 3. Egg production response to peptide supplementation

	Items	Peptide, g kg^{-1}				Pooled SEM	P-value
		0	2	4	8		
Wk 0 to 4	Egg mass, g/hen daily	55.7	55.7	56.7	56.3	0.525	0.462
	Egg weight, g	57.1	57.2	57.7	57.2	0.239	0.232
	Hen-day production, %	95.3	95.0	95.5	96.2	0.760	0.712
Wk 4 to 8	Egg mass, g/hen daily	57.6	58.5	58.5	58.5	0.590	0.647
	Egg weight, g	60.6	60.7	60.6	60.6	0.234	0.989
	Hen-day production, %	95.2	96.8	96.6	97.0	0.745	0.369
Wk 0 to 8	Egg weight, g	58.8	58.8	59.1	58.8	0.166	0.383
	Hen-day production, %	95.2	96.3	96	96.5	0.489	0.282

Table 4. Performance response to peptide supplementation in laying hens

Items		Peptide, g kg^{-1}				Pooled	P-value	Contrasts	
		0	2	4	8	SEM		Linear	Quadratic
Wk 0 to 4	ADFI	117.3	119.1	118.6	117.7	0.643	0.198		
	FCR	2.21	2.24	2.20	2.20	0.019	0.280		
Wk 4 to 8	ADFI	117.3	121.1	126.3	118.4	2.952	0.167		
	FCR	2.06	2.11	2.19	2.04	0.058	0.289		
Wk 0 to 8	ADFI	117.5	119.4	122.4	117.9	1.509	0.116		
	FCR	2.14	2.17	2.19	2.12	0.027	0.194		
	Final body weight, kg	1.93	2.01	2.01	1.97	0.021	0.050	0.538	0.011

ADFI – daily feed intake, g bird^{-1}.

FCR – feed conversion ratio.

Table 5. Internal quality of the eggs collected after 4 weeks of feeding peptide to laying hens

	Items	Peptide, g kg^{-1}				Pooled SEM	P values
		0	2	4	8		
Wk 4, d 0	Albumen height, mm	11.7	11.9	11.9	11.9	0.174	0.966
	Haugh unit	106.4	107.2	106.8	106.7	0.657	0.848
	Yolk width, mm	38.1	37.3	37.7	37.6	0.302	0.278
	Yolk height, mm	18.9	19.4	19.5	19.6	0.363	0.566
	Yolk index	0.99	1.04	1.03	1.04	0.018	0.201
	Yolk colour	10.1	10.2	10.1	9.51	0.207	0.124
Wk 4, d 14	Albumen height, mm	8.08	8.69	9.12	8.54	0.336	0.939
	Haugh unit	89.8	93.0	94.5	91.7	1.735	0.980
	Yolk width, mm	35.9	35.8	36.0	35.5	0.332	0.233
	Yolk height, mm	15.9	16.5	16.5	16.2	0.222	0.152
	Yolk index	0.89	0.92	0.92	0.92	0.013	0.760
	Yolk colour	9.20	9.20	9.53	9.23	0.188	0.098

Albumen CP*, g kg^{-1}	859	859	862	861	2.90	0.860

* Crude protein expressed on dry matter content.

Table 6. Internal quality of the eggs collected after 8 weeks of feeding peptide to laying hens

	Items	Peptide, g kg^{-1}				Pooled	P-value	Contrasts	
		0	2	4	8	SEM		Linear	Quadratic
Week 8, d 0	Albumen height, mm	11.6	11.8	11.9	12.2	0.246	0.282		
	Haugh unit	104.7	106.2	106.2	107.6	1.070	0.291		
	Yolk width, mm	35.5	35.9	35.7	35.9	0.400	0.912		
	Yolk height, mm	18.0	18.0	18.0	18.4	0.343	0.606		
	Yolk index	0.993	0.996	1.030	1.030	0.019	0.656		
	Yolk colour	9.71	9.40	9.34	10.00	0.217	0.481		
Week 8, d 14	Albumen height, mm	6.87	7.09	7.03	7.12	0.176	0.029	0.020	0.046
	Haugh unit	81.9	83.5	82.8	83.3	1.064	0.015	0.005	0.097
	Yolk width, mm	36.7	36.6	35.6	36.2	0.451	0.033	0.004	0.760
	Yolk height, mm	20.2	20.4	20.1	20.5	0.340	0.857		
	Yolk index	1.10	1.12	1.13	1.13	0.020	0.047	0.006	0.975
	Yolk colour	8.70	8.50	8.70	8.80	0.156	0.296		

Albumen CP*, g kg^{-1}	868	870	869	870	1.86	0.850

* Crude protein expressed on dry matter content.